Effect of trypsin treatment of bacteriorhodopsin on its orientation in reconstituted vesicles

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Effect of trypsin treatment of bacteriorhodopsin on its orientation in reconstituted vesicles

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The light-induced proton pump bacteriorhodopsin (bR) can be used to generate an electrochemical proton gradient (Δp) across the membrane of reconstituted vesicles. bR can be incorporated into membrane vesicles derived from the lactic acid bacterium *Streptococcus cremoris* by fusion of these bacterial membrane vesicles with bR-containing proteoliposomes (Driessen et al., 1985). For studies on the relationship between the Δp and solute transport (e.g. amino acids) in such model systems, a Δp, inside alkaline and negative, is required. Only one method has been described for the reconstitution of bR proteoliposomes that show light-dependent proton extrusion (Happe et al., 1977; Hellingwerf, 1982) and consequently generate a rightside-out-oriented Δp. These rightside-out bR proteoliposomes can be obtained by co-sonication of purple membranes together with phospholipid vesicles, containing acidic phospholipids using a short acidic stage (pH 2.4–3.0) during sonication. Although these preparations show light-dependent proton extrusion, a considerable part of the vesicles contains bR with an inside-out orientation.

It has been suggested (Hellingwerf, 1982) that the distribution of charges over the two sides of bR is an important factor in determining the orientation of the protein in the reconstituted membrane. We therefore studied the effect of proteolytic treatment of bR on the orientation of the protein in the reconstituted membrane. Under fully native conditions only the C-terminal part of the protein is accessible to proteolytic enzymes (e.g. trypsine). A peptide fragment is removed containing five carboxylic acid groups, negatively charged at neutral pH. Removal of this peptide results in a decrease of the negative surface charge density of the C-side (cytoplasmic side) of bR (Arrio et al., 1984) resulting in an extensive aggregation of the purple membrane sheets.

Trypsin-treated bR was reconstituted into phospholipid vesicles, containing the negatively charged phospholipid cardiolipin, by the use of the rightside-out reconstitution procedure (Happe et al., 1977; Hellingwerf, 1982) at different pH values during the acid stage. An inversion of the direction of proton translocation upon illumination can be observed when the pH of the acid stage of the reconstitution procedure is varied. This inflection point is around pH 3.0 for non-treated bR and about 3.5 for trypsin-treated bR. Trypsin-treated bR can be reconstituted in a rightside-out orientation with a higher efficiency, compared to non-treated bR. This is concluded from the following observations: (i) the extent of proton extrusion by trypsin-treated bR proteoliposomes is considerably higher than by non-treated bR, (ii) the proton extrusion extent is stimulated 1.2–2-fold by valinomycin and this is not observed in non-treated bR, (iii) tetraphenylphosphonium (TPP^+) is accumulated and tetraphenylboron (TPB^-) is extruded by proteoliposomes prepared with trypsin-treated bR. Proteoliposomes prepared with native bR show some TPB^- uptake, indicating the presence of proteoliposomes generating a Δp, interior positive and acid.

These results support the mechanism proposed by Hellingwerf (1982) for the reconstitution of bR with an inside-out orientation in which the charge distribution over the two sides of bR plays an important role.

The use of bR proteoliposomes with an inside-out orientation may be an important factor for a successful incorporation of bR into bacterial membrane vesicles, such that a Δp, inside negative and alkaline, is generated upon illumination.

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Direct interaction between electron transfer chains and solute transport systems in bacteria

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In studies on solute transport in *Rhodopseudomonas sphaeroides* we have demonstrated that in addition to a proton motive force cyclic or linear electron transfer is required for solute transport. At constant proton motive force values the uptake of solutes is a linear function of the rate of cyclic or linear electron transfer (Elferink, 1983*a*, 1984). This is also valid for the lactose transport protein from *Escherichia coli* introduced in the cytoplasmic membrane of the phototrophic bacterium via genetic manipulation (Elferink, 1983*b*).

Regulation of solute uptake by electron transfer through a linear electron transfer chain is also observed in *E. coli*. In intact cells of *E. coli* transport of lactose glutamate and TMG, a melibiose analogue, was studied. For glutamate uptake, in addition to a proton motive force, respiration is obligatory, like for solute transport in *Rps. sphaeroides*. On the other hand, lactose uptake takes place at anaerobic conditions, but initiation of linear electron transfer results in higher initial uptake rates and the H^+/lactose stoichiometry increases from 1 to 2. The interaction between the electron transfer chains and transport carriers is not restricted to H^+/symport systems but also occurs in Na^+/TMG symport via the melibiose transport carrier.

In cytoplasmic membrane vesicles of *E. coli* the rate of lactose uptake correlated with the rate of linear electron transfer when the vesicles were energized with D-lactate or with glucose/PQQ. In contrast, when the vesicles were energized with succinate no influence of electron transfer activity on lactose transport activity was observed. On the basis of these observations a model for solute transport is postulated in which the carrier is depicted as a non-obligatory intermediate at an electronegative level of the electron transfer system (Konings et al., 1984).

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